# VITELLOGENIN DISPARITY IN *PHORMIA REGINA* (DIPTERA: CALLIPHORIDAE)[1]

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ABSTRACT: In *Phormia regina,* female fat body was stimulated to provide for normal vitellogenesis by hormones from either male or female corpora allata. Male fat body failed to provide that synthetic support even though exposed to female hormones by glandular transplants and intersex parabiosis. The present results, from an *in vivo* oocyte assay with natural hormone sources, are consistent with earlier data (obtained with synthetic hormone and electrophoretic assay) which suggest that a sexual disparity in fat body responsiveness (rather than a disparity in circulating hormones) accounts for the disparity in vitellogenin production by the sexes of this species.

Insect follicles sequester selected hemolymph proteins (vitellogenins) and concentrate them in the yolk of developing oocytes (Hagedorn and Kunkel 1979; Bownes 1986). The vitellogenins, synthesized by fat body and released into circulation, are major hemolymph constituents in many female insects, but are minor or absent in the male circulation (Hagedorn and Kunkel 1979). The blowfly, *Phormia regina,* conforms to that generalization (Mjeni and Morrison 1973). In *Phormia,* as in many other insects (Bownes 1986), juvenile hormone from the corpus allatum drives the selective synthesis of vitellogenins used in vitellogenesis (yolk deposition) (Mjeni and Morrison 1973). Sexual disparity in vitellogenin production emanates from gender difference in circulating hormones, as in some vertebrates (Wallace and Bergink 1974), or from disparate synthetic responses by male and female tissue to the same hormone. For *Phormia* the former seems unlikely: typical vitellogenin disparity develops between allatectomized males and females when treated with the same analog of juvenile hormone (Mjeni and Morrison 1976). The present study tests that electrophoretic evidence obtained with synthetic hormone (Mjeni and Morrison 1976) through very different *in vivo* techniques that employ natural hormone sources.

## MATERIALS AND METHODS

Each experiment used a cohort of flies that emerged, and were segregated by sex, during a two hour span, and fed a protein-free diet of 0.1M sucrose *ad libitum* till surgery on the fourth day after eclosion. *Ad lib.* access to selected diets was given during each ensuing experiment. Rearing methods were after Belzer (1978), allatectomy and shams after Thomsen (1942), and anesthesia

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was $CO_2$. Corpora allata from males were transplanted into allatectomized females by grasping the donor gland with fine forceps and implanting it into a hole cut in the recipient's thorax. The cuticle was replaced and waxed to prevent loss of the transplant. Parabiotic flies, whose hemolymph exchange is through a thoracic nexus (Green 1964), were prepared according to Dethier, Solomon and Turner (1965). Females, with mouths waxed shut to preclude feeding, were placed in parabiosis with either a male or female that would subsequently be permitted to feed *ad lib.* on protein (0.1M sucrose brought up to 20% (wt/vol) yeast extract). Each feeding member of a parabiotic pair received three implanted female corpora allata when parabiosis was established. Feeding solutions were prepared and changed daily. Inspection, through a dissecting microscope, of the progress of vitellogenesis in exposed ovaries *in situ* served as an assay of prior vitellogenin synthesis (cf. Roth and Porter 1964; Wallace and Bergink 1974); a calibrated ocular allowed measurement of occyte length and yolk accumulation. Experimental conditions were constant 3200 luces, 65% relative humidity, and 24°C. Data were statistically analyzed by the Mann-Whitney U test (Siegel 1956).

## RESULTS

Terminal oocyte length in newly emerged females measured 0.04mm (Fig. 1a). After 14 days of protein-free diet, none exceeded 0.14mm (Fig. 1b). Preliminary studies established that yolk is first visible in the posterior pole when terminal oocyte length reaches 0.21mm; mature oocytes are 1.05mm long and filled with dense yolk. *Ad lib.* protein feeding supported full maturation of oocytes in normal females (Fig. 1c; cf. Belzer 1978) but not in allatectomized females (Fig. 1d). Neither sham surgery (Fig. 1e), nor allatectomy followed by implantation of a male corpus allatum (Fig. 1f), impaired oocyte development with this diet. Two of those allatectomized females, with male corpus allatum, were spared and mated; each deposited viable eggs two days later. Feeding female members of parabiotic pairs managed egg development that was statistically indistinguishable from normal females' (cf. Fig. 1g and 1c). Their aphagic female parabionts (Fig. 1h) managed significantly less ($p = 0.05$) oocyte growth (cf. Fig. 1h and 1g), but in all oocytes that exceeded 0.21mm the proportion and density of yolk deposition that was achieved was indistinguishable from that in eggs of normal protein-eating females that were sacrificed when oocytes were of comparable lengths (cf. Fig. 1h and 1i). Oocyte growth in aphagic females in parabiosis with feeding males (Fig. 1j) was significantly less ($p = 0.01$) than that occuring in aphagic females in parabiosis with feeding females (Fig. 1h); more pertinently, yolk deposition in oocytes that exceeded 0.21mm in the former was virtually nonexistent and significantly less (p

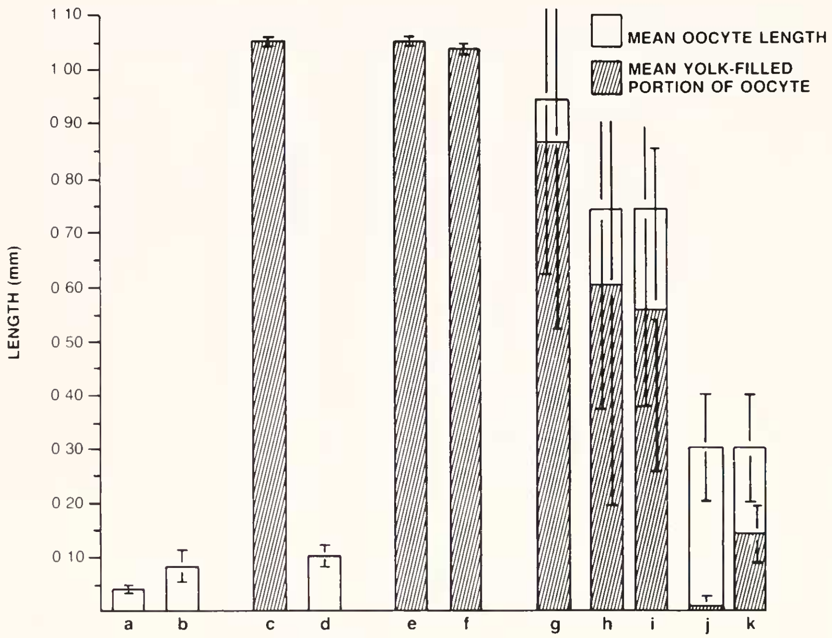


Figure 1. Terminal Oocyte Length and Yolk Accumulation in Various Female *Phormia;* bars = S.D.

(a) Newly emerged, N = 20. (b) Unoperated, fed only sucrose, N = 20, day 14. (c) Unoperated, fed protein, N = 20, day 10. (d) Allatectomized, fed protein, N = 20, day 10. (e) Sham allatectomy, fed protein, N = 20, day 10. (f) Allatectomized with implanted male corpus allatum, fed protein, N = 18, day 10. (g) Feeding member of parabiotic pair, N = 20, day 14. (h) Aphagic member in parabiosis with feeding female of group g, N = 17, day 14. (i) Unoperated, fed protein, N = 10, sacrificed when oocytes reached lengths comparable to group h. (j) Aphagic member in parabiosis with feeding male, N = 7, day 14. (k) Unoperated, fed protein, N = 10, sacrificed when oocytes reached lengths comparable to group j.

= 0.001) than the amount of yolk deposited in oocytes of comparable size in normal protein-eating females (cf. Fig. 1j and 1k).

## DISCUSSION

In parabiotic pairs, the fat body of the feeding member should dominate the types of proteins synthesized and released into circulation, by virtue of its preferential access to dietary amino acids. Of circulating proteins, it is the sequestered vitellogenin that provides the dense reflective appearance

of dipteran yolk (Roth and Porter 1964; cf. Wallace and Bergink 1974). While fat body of female flies in the present experiments provisioned the oocytes of aphagic female parabionts with dense yolk deposits, the fat body of males failed to do so, even though both were exposed to hormones from implanted female corpora allata and hemolymph exchanged from female parabionts (see studies on dye and isotope transport between parabiotic flies - Green 1964). Incidentally, vitellogenesis in aphagic females, relying on feeding parabiotic males for their hemolymph proteins, could not be improved by implanting into the males several corpora allata, or corpus allatum-cardiacum complexes, from protein-fed females entering various stages of vitellogenesis (Belzer, unpublished). Thus, while natural hormones seem interchangeable between the sexes (as evidenced by the normal vitellogenesis, including oviposition of viable eggs, achieved in allatectomized females with a male corpus allatum transplant), the fat bodies of the two sexes seem to differ in their synthetic responsiveness to female hormones.

Sexual disparity in a tissue's metabolic response to a sexually neutral hormone is common among animals, and it has been demonstrated *in vitro* for primary tissue cultures of cockroach, mosquito and locust fat body (Wyss-Huber and Luscher 1972; Hagedorn and Kunkel 1979). The results of experiments with juvenile hormone analog (Mjeni and Morrison 1976), and with natural hormone in the present study, are consistent with such a circumstance in *Phormia*. The present study helps to allay possible concern that results obtained with hormone analog could have been artifactitious.

While the results of these two diverse approaches to the problem are consistent with one another, potential for uncontrolled variables (such as unknown hormone interactions) that are ever present in the *in vivo* systems of these two approaches argues for the development of purified fat body cell cultures (as envisioned by Hagedorn and Kunkel 1979) so that more completely controlled *in vitro* studies might be designed to further assess and characterize synthetic parameters of fat body in *Phormia*.

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This latest volume of a continuing series contains five contributions on calcium regulation, midgut function, reabsorption in hindgut, proctolin, and regulation of successive steps in insect reproduction.

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The Hope Entomological Collections are of international importance. This book traces the history of the collections up to the present time and provides a comprehensive list of donors and of archives in the Hope library.

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356 previously unpublished drawings of high scientific and artistic quality, of representative species of biting flies. Species included are the sandfly, *Phlebotomus papatasi;* the mosquito, *Aedes aegypti;* the blackfly, *Simulium* sp.; the deerfly, *Chrysops caecutiens;* and the stablefly, *Stomoxys calcitrans.*